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EFFECTS PRODUCED BY CUTTING PARAMECIUM CELLS.

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CONTENTS.

In a previous paper on *Uronychia* I have shown that the power of regeneration in a hypotrichous ciliate is a factor of cell age, increasing as the cell grows older after division, until a maximum power is attained just prior to cell division. In the present paper I desire to describe the results obtained by similar methods of experimentation on a much more difficult subject, the holotrichous ciliate *Paramecium*. Here in forms from ordinary cultures, the power of regeneration is very poorly developed and the nuclear apparatus is more concentrated. Similar experiments on *Paramecium* have often been attempted, but the difficulties in technique are so great that, with the exception of Balbiani's beautiful work in 1893, little or no extensive work has been done. The endeavor to ascertain what happens in a cell in which the power of regeneration is slight and when the physico-chemical equilibrium is suddenly disturbed is a problem well worth the patience and repeated failures necessary in getting the small percentage of success, and the results obtained at intervals during the last three years are here brought together for the first time. In a subsequent paper I shall publish my results with another race of *Paramecium caudatum* on regeneration in relation to cell division.

Material and Method.

Of the thousands of *Paramecium* that I have succeeded in cutting with a scalpel as in the *Uronychia* work, only about two hundred, of which one hundred and fifty approximately are here described, have given complete records. The cell to be operated is placed on a glass slide with a drop of water of minimum size and the cell is cut with a fine scalpel under a binocular microscope. It is a comparatively simple matter to actually cut the cell in two parts but if the knife passes through the macronucleus or its membrane, the fragments are useless for the nucleus is never retained and both parts quickly collapse. The results show a marvellously delicate coördination of the parts of the cell, a very slight injury, as the following records will show, throwing the physiological mechanism out of gear.

Immediately after cutting a cell the peristome depression fills out, leaving the cell periphery perfectly smooth; the mouth cavity, however, does not disappear but persists as a miniature cave in the protoplasm. In from fifteen minutes to half an hour after the operation the part remaining of the old peristome reappears, movements of translation and rotation are continued as before, but in most cases the cell is truncated and the cut surface appears as clean cut and smooth as though a wax model of *Paramecium* had been cut with a sharp knife. If the cut cell lived for four hours after the operation it was recorded as a successful experiment and the results here described were made on cells that invariably recovered from the shock of the operation and lived at least four hours afterwards. Fragments living after this four hour test were kept isolated in small watch glass culture dishes in a moist chamber. The culture fluid was 24-hour-old hay tea made by boiling a small quantity of hay in tap water. The division rate of normal *Paramecium* kept under similar conditions varied from one to three divisions per day according to the race under observation.

In the case of *Uronychia* both fragments of the cell resulting from a single cut, continue to live for at least 24 hours, and frequently such fragments continue to live for three or four days without regeneration. With *Paramecium*, on the other hand, the smaller fragment almost always collapses. One or two

exceptional cases of continued activity may be mentioned here. One, a giant form, was cut through the mouth at 11.15 A.M. At 4 P.M. both fragments were alive, the posterior fragment swimming actively with the truncate end in advance, the anterior fragment with the truncate end behind. On the following day the smaller posterior fragment was dead, the other, anterior, lived for 72 hours when it was killed (no. 6). A second case, cut in the same place, resulted in two fragments both of which were alive after six hours. On the following day the smaller one was dead but the other lived and gave rise to two types of cells, one much smaller than the other and with a blunt posterior end, while the other had the characteristic pointed end of *Paramecium caudatum*. In still another case the giant form was cut posterior to the mouth, the posterior fragment being about one half the size of the anterior part. Both parts were alive after six hours but both were dead on the following day.

In all of these cases the organisms were treated for from fifteen minutes to one half an hour with dilute neutral red, granules in the cell being deeply stained at the time of cutting. What effect the neutral red has upon the consistency of the protoplasm I do not know, but certainly there was a marked difference in the resistance to collapse after treating with this dye. In only two other cases have I succeeded in keeping both fragments alive after the operation on normal forms; in one case the smaller fragment lived only ten minutes after the operation; in the other case (Table II., no. 63) the original cell was treated with nuclein before cutting, and both parts lived 24 hours. In the majority of cases the smaller fragment collapses immediately after removal of the knife.

Several different races of *Paramecium* have been used, but giant forms on the whole have been selected because of the greater ease of cutting. The smaller races and races of *Paramecium aurelia* do not allow sufficient play for the knife edge and too great a zone is crushed by the operation. One race of giant forms was obtained from brackish water at Roscoff, France, for which I am indebted to Mlle. Lipska. The other races have been obtained from time to time from wild cultures in the laboratory at Columbia University. As there is little or no

difference in the percentage of successful results in the different races I shall not particularize but will deal with them all as of one race. Up to the present in these experiments, no systematic effort has been made to study the regenerative power at different periods between division phases, as in the studies on *Uronychia*. The percentage of cases of regeneration was so small that such a study seemed valueless, but with a race now under experimentation more satisfactory results are expected. Of the seven cases of regeneration obtained in the one hundred and forty-nine cases here recorded, five were cells that were cut while either dividing or conjugating, results indicating that not only division age but racial age is also a potent factor in regeneration. Further work on this phase of the subject is now under way and will undoubtedly yield interesting results.

In the following tables the experiments are grouped according to the region of the cell originally cut. The cell is by no means homogeneous and the different portions are not equipotent. If we imagine the *Paramecium* long diameter to be divided into four equal parts by three cuts, the central one would pass through the plane of division of the cell, the upper one would divide the anterior half into two dissimilar quarters, the lower one would divide the posterior half of the *Paramecium* into dissimilar quarters. In the tables, the zones indicating these several quarters are numbered from one to four, 1 being the most anterior, 2, the next anterior, 3 the posterior central zone and 4 the terminal posterior zone (text figure 1). The most important organs of the cell are contained in zones 2 and 3, zone 2 containing the macro- and micronucleus, and zone 3 containing the mouth. Zones 1 and 4 do not contain any of the more important organs, and yet, as the sequel shows, these parts if injured are rarely replaced and their absence has a remarkable effect on further activities of the cell.

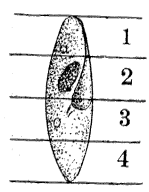

FIG. 1.

TABLE I.

Paramecium caudatum CUT IN ZONE I.

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	Active. No regeneration.	Divided. One truncate, one normal.	Normal divided, other not regenerated.	Killed.
No. 2	No regeneration.	Dead. No regeneration		Conjugating when cut.
No. 3	Two normal cells. One small.	Small one dead, other dividing.		Dividing when cut.
No. 4	No regeneration.	No regeneration.	No regeneration. Killed.	Fig. 2, Plate I.
No. 5	No regeneration.	No regeneration. Control divided.	No regeneration.	
No. 6	Cut end ciliated.	Divided twice, four normal cells.		
No. 7	Dead.			
No. 8	Dead.			
No. 9	Dead.			
No. 10	Dead.			
No. 11	Dead.			Fig. 1, Plate I.
No. 12	Dead.			
No. 13	No regeneration.	Dead.		
No. 14	No regeneration.	Dead.		
No. 15	Dead.			
No. 16	Small, sluggish, deformed.	Dead.		
No. 17	No regeneration.	Dead.		
No. 18	Dead.			
No. 19	Dead.			
No. 20	No regeneration.	Divided. One small. Rounded ends.	Both alive and divided (see description).	

TABLE II.

Paramecium caudatum CUT IN ZONE II.

Expt.	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	Unhappy. No regeneration.	Sluggish, no regeneration. Killed.		No nucleus in cell.
No. 2	No regeneration.	No regeneration.	No regeneration.	Conjugating when cut. See p. 49.
No. 3	Cell divided in original plane.	Both cells divided once.	No regener. Same as before.	Cell in conjugation when cut. Fig 3, Plate I.
No. 4	Normal posterior. Abnormal anterior divided in original plane.	Both cells normal. Regeneration perfect.	Both dead.	
No. 5	Dead. No regeneration.			
No. 6	Monster. 2 mouths.	Monster.	Monster.	Killed 96 hours. See p. 53. Fig. 18, Plate III.
No. 7	Dividing in orig. center. Killed.			Fig. 4, Plate I.
No. 8	Dividing.	Both dead.		
No. 9	Divided in orig. center.	No regeneration. Killed.		Fig. 5, Plate I. See p. 49.
No. 10	No regeneration.	Dead.		
No. 11	Normal.	Normal.		Dividing when cut. Fig. 10, Plate I.
No. 12	Dead.			
No. 13	Dead.			
No. 14	No regeneration.	Left in culture. 10 days later both large and small forms in culture dish. See p. 51.		
No. 15	Rounded anteriorly. No growth.	Dead.		Neutral red.
No. 16	Dead.			
No. 17	Dead.			
No. 18	Dead.			Neutral red one half hour.
No. 19	Dead.			
No. 20	Dead.			
No. 21	No regeneration.	Divided in original plane.	Both dead.	Fig. 6, Plate I.
No. 22	Divided in original plane.	No regeneration.	Monster.	See p. 56. Fig. 16, Plate II.
No. 23	Dead.			
No. 24	Dead.			

TABLE II.—*Continued.*

Expt.	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 25	Dividing in original plane.	No regeneration. Monster.	Monster.	See p. 57. Fig. 20, Plate III.
No. 26	Dead.			
No. 27	Dead.			
No. 28	Dead.			
No. 29	Dividing in original plane.	Dead.		Fig. 8, Plate I.
No. 30	Dead.			
No. 31	Dead.			
No. 32	Dead.			
No. 33	Dead.			
No. 34	Dead.			
No. 35	Divided in original plane.	Monster.	Monster.	See p. 54.
No. 36	Dead.			
No. 37	Dead.			
No. 38	Dead.			
No. 39	Divided in original plane.	Monster.	Monster.	See p. 54.
No. 40	Divided in original plane.	Monster.	Monster.	See p. 55. Fig. 17, Plate II.
No. 41	Dead.			
No. 42	Dead.			
No. 43	Dead.			
No. 44	Dead.			
No. 45	Dead.			
No. 46	Dead.			
No. 47	Dead.			
No. 48	Dead.			
No. 49	Alive. No regeneration.	Dead.		
No. 50	Dead.			
No. 51	Divided in original plane.	Monster.	Monster.	See p. 54. Fig. 14, Plate II.
No. 52	Dead.			
No. 53	Dead.			
No. 54	Large. No regeneration.	Larger. No regeneration.	Very large. No regeneration.	Divided in 96 hours. See p. 50. Fig. 7, Plate I.

TABLE II.—*Continued.*

Expt.	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 55	Dead.			
No. 56	Dead.			
No. 57	Lively. No regeneration.	Dead.		
No. 58	No regeneration.	Dead.		
No. 60	Active. No regeneration.	Dead.		
No. 61	Dead.			
No. 62	Very weak.	Dead.		
No. 63	Both fragments alive and active.	Dead.		
No. 64	No regeneration.	Dead.		
No. 65	Dead.			
No. 66	Dead.			
No. 67	No regeneration.	No regeneration.	Dead.	
No. 68	Larger. No regeneration.	Larger. No regeneration.	Monster.	See p. 54.

TABLE III.

Paramecium CUT IN ZONE III.

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	No regeneration.	No regeneration.	No regeneration.	See p. 51.
No. 2	Both cells truncate. No regeneration.	Both dead.		Conjugating when cut.
No. 3	No regeneration.	No regeneration.	No regeneration.	Ex-conjugant. Killed 96 hours.
No. 4	No regeneration.	No regeneration.	No regeneration.	Killed 96 hours ; ex-conjugant. Fig. 12, Plate I.
No. 5	Dividing in original plane.	Dead.		
No. 6	No regeneration.	Monster. 2 mouths.	Monster. Killed.	See p. 54. Fig. 19, Plate III.
No. 7	Large. No regeneration.	Larger. No regeneration.	Very large. No regeneration.	Killed 96 hours. See p. 52.
No. 8	Dead. Cells united.			Conjugating when cut.
No. 9	Two truncated cells.	No regeneration.	No regeneration.	Both dead 96 hours. No regeneration. Conjugating when cut.
No. 10	No regeneration.	No regeneration.	No regeneration.	Dead 96 hours. No regeneration.
No. 11	No regeneration.	Monster. 2 mouths.	Monster.	See p. 54.
No. 12	No regeneration.	Very large, no regeneration.	Divided, one normal, other abnormal.	Killed 96 hours.
No. 13	No regeneration.	No regeneration.	No regeneration.	Killed 96 hours. See p. 51.
No. 14	Dead.			
No. 15	Dead.			
No. 16	Dead.			
No. 17	Divided in original plane.	Very large.	Monster.	See p. 55. Fig. 15, Plate II.
No. 18	Dead.			
No. 19	Huge. No regeneration.	Divided in original plane.	Abnormal, cell very large.	See p. 52. Fig. 9, Plate I.
No. 20	Dead.			
No. 21	Dead.			
No. 22	No regeneration.	Dead.		
No. 23	No regeneration.	No regeneration.	Monster.	See p. 54. Fig. 13, Plate II.
No. 24	Divided, both dead.			
No. 25	Dead.			
No. 26	Dead.			

TABLE III.—*Continued.*

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 27	Dead.			
No. 28	Dead.			
No. 29	Dead.			
No. 30	Dead.			
No. 31	Dead.			
No. 32	Dead.			
No. 33	Dead.			
No. 34	No regeneration.	No regeneration.	Dead.	
No. 35	Dead.			
No. 36	Dead.			
No. 37	Dead.			
No. 38	No regeneration.	Dead.		
No. 39	No regeneration.	Dead.		
No. 40	Dead.			
No. 41	No regeneration.	Monster.	Monster.	See p. 54.

TABLE IV.

Paramecium caudatum CUT IN ZONE 4.

	Condition after 24 Hours.	Condition after 48 Hours.	Condition after 72 Hours.	Remarks.
No. 1	Regeneration perfect.	Normal. Divided.	One weak, other cut.	Dead.
No. 2	No regeneration.	Dead.		
No. 3	Regeneration perfect. Cells very small.	Normal.	Normal.	Conjugating when cut. See p. 53.
No. 4	Both dead.			Conjugating when cut.
No. 5	Slightly truncate, crystals in anterior end.	Same.	Dead.	Cut during division. Fig. 11, Plate I.
No. 6	Dead.			
No. 7	Dividing but cannot separate.	Separated but dead.		
No. 8	Dead.			
No. 9	Dead.			
No. 10	Dead.			
No. 11	Dead.			
No. 12	No regeneration.	No regeneration.	No regeneration.	Dead after 120 hours.
No. 13	No regeneration.	Dead.		
No. 14	Dead.			
No. 15	Divided, one normal, other truncate.	Both dead.		
No. 16	No regeneration.	No regeneration.	No regeneration.	By fifth day divided twice, all normal.
No. 17	Dead.			
No. 18	Dead.			
No. 19	Dead.			
No. 20	Dead.			

TABLE V.

SUMMARY OF TABLES I.–IV.

Zone of Cut.	No. of Cases.	Alive after 24 Hours.		Alive after 48 Hours.		Alive after 72 Hours.		Monsters.		Regeneration.	
		No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
1	20	11	55.5	4	20.	2	10.	0	0	2	10.
2	68	29	42.6	14	20.6	11	16.2	8	11.7	3	4.4
3	41	20	48.8	15	36.6	14	34.1	5	12.2	0	0
4	20	8	40.	4	20.	4	20.	0	0	2	10.
Total	149	68	45.6	37	25	31	20.8	13	8.7	7	4.7

2. ANALYSIS OF TABLES AND EXPERIMENTS.

1. *Analysis of Table I.*

In the experiments included in this table the cells were cut in zone 1 or through the anterior quarter of the cell, a part containing only the anterior terminus of the peristome but none of the important cell organs.

Of the 20 cases recorded as having lived at least four hours after the operation, some (9) died within 24 hours; others (5) died without regeneration or division within 48 hours; others (3) lived for 72 hours or longer without regeneration; others (2) ultimately gave rise to normal descendants, and one divided within 24 hours giving rise to normal cells.

Number 11 is a good example of the 9 cases of death within 24 hours. Here a clean cut resulted in an anterior small fragment which instantly collapsed, and a larger rounded fragment in which the peristome was obliterated. Within half an hour the peristome reappeared exactly as it was before except for the small part removed (Fig. 1). No rearrangement of the protoplasmic material occurred and the old form was perfect except for the missing part. So far as external appearances were concerned the cell should have lived.

Of the five specimens that died within the next 24 hours, number 16 is a good example. Here no effort was made to regenerate and at the end of 24 hours the fragment was small and sluggish with the remainder of the peristome perfect. The cell died before the end of the next 24 hours.

Number 4 is a good example of a fragment that lived for 73 hours without regeneration or division. The cut surface had

developed cilia and regeneration had progressed to that extent but no further (Fig. 2).

The two cases of regeneration were 3 and 6, only one of which was a vegetative form, the former being cut while dividing the latter during a vegetative stage. Number 3 was first cut in the plane of division separating the two cells; one of these was then cut again in the first zone. On the following day both cells were normal *P. caudatum*, but the cell that had been cut was much smaller than the sister cell. The cut one died on the third day.

The vegetative form that regenerated showed after 24 hours an anterior end somewhat blunt but rounded and ciliated, and on the following day it had divided twice, giving rise to four cells which could not be distinguished from normal cells. The double division indicates that the original cell was about ready to divide at the time of cutting and that removal of the anterior part had no effect on the subsequent divisions.

Analysis of Table II.

The mortality of *Paramecium* cut through zone 2 is considerably greater than that for zone 1. One chief reason for this is probably the fact of the injury or destruction of the macronucleus which usually occupies this region. Of the 68 cases 47.4 per cent. died before the end of the first 24 hours; 79.4 per cent. were dead before the end of 48 hours; but if the fragments lived through this period there was a good chance of continued life for some days, at 72 hours for example there were only 83.8 per cent. dead, only a slight increase over the mortality at the end of 48 hours. The majority of these or 8 out of 11 were monsters while 3 out of the 68 regenerated into perfect cells.

Of those that lived 48 hours or more after cutting, some (5) failed to divide or else formed monsters; others divided in the plane of the original center of the cell giving rise to (*a*) cells with reduced vitality which soon died; (*b*) one normal cell and one abnormal which died; (*c*) one normal cell and one abnormal which divided more than once; (*d*) one normal and one abnormal cell which formed a monster, and (*e*) two normal cells.

Of the four cases living 48 hours or more in which no division occurred, the history was varied; the experiments were numbers

1, 2, 67 and 68 of Table II. Number 1 was killed after 48 hours and found to have only a few nuclear fragments. Number 2 was killed at the end of 96 hours, and, although cut during the process of conjugation, was found to have a normal macronucleus. Number 67 was dead at the end of 72 hours, no regeneration occurred but the cell was very lively and examination showed a normal macronucleus. Number 68 was a vegetative form which recovered perfectly from the shock of the operation and lived without regeneration or division for a period of three days.

Of the cases in which the cut fragment divided in the original central plane of the cell, the dividing fragment was killed in two cases for the determination of the nuclear conditions (*a*); both daughter cells died in 3 cases (*b*); an abnormal cell was formed which divided three times in one case (*c*); monsters were formed in 7 cases (*d*); and regeneration occurred in 3 cases.

(*a*) The dividing fragment was killed either during division or immediately afterwards to determine the conditions of the nuclei in experiments 7 and 9. Number 7 is shown in Fig. 4. The posterior cell (4, *c*) is apparently normal, the micronucleus is dividing normally but the macronucleus is somewhat distorted. Number 9 was evidently an ex-conjugant when operated and dividing for the second time. It was killed 24 hours after division; the abnormal cell had one of the four new macronuclei and several of the degenerating fragments of the old macronucleus; the other cell was a normal exconjugant (Fig. 5, Plate I.).

(*b*) Both daughter cells died without regeneration in experiments 8, 21 and 29. Number 21 is a good example of these cases. Here the cell was cut as shown in Fig. 6, *a*. On the following day there was a small tentacle-like appendage on the cut surface (6, *b*). On the second day the fragment had divided through the original center of the cell into dissimilar daughter cells (6, *c*), one of which was normal in shape, but with a collection of black granules at the anterior end (6, *e*); the other cell was small and as sharply truncated as when cut (6, *d*). On the third day both were dead.

Number 29 was similar in history but the abnormal fragment formed by division of the cut cell was extremely minute, being little more than a small sphere with a nucleus and a contractile

vacuole. Its small size was due to the proximity of the cutting plane to that of the normal division plane of the cell. It died on the second day after cutting (Plate I., Fig. 8, *a*, *b*, *c*, *d*).

(*c*) *Division of the Abnormal Cell.*—In one experiment of this series (no. 54) the abnormal cell, resulting from the division of the original cut fragment, divided three times. The vegetative cell was cut as shown in Fig. 7, *a*. After 24 hours the fragment was lively and complete as far as the plane of the cut which was sharply marked (Fig. 7, *b*). After 48 hours it had grown much larger and was particularly broad at the anterior or truncate end. After 96 hours it had divided into an abnormal truncate cell (7, *c*, and 7, *d*) and a normal cell (7, *e*). On the sixth day this abnormal cell had grown in size but retained the original truncate anterior end (7, *f*) while the normal cell had divided to form two normal cells of which one was cut (see Table III., no. 38) and the other lost in cutting. On the seventh and eighth days the abnormal cell had grown very large again and on the ninth day divided to form two small dissimilar cells (7, *g*, *h*), each, however, with only one vacuole. On the twelfth day each of these divided once more giving rise to four small abnormal cells (7, *i*, *j*, *k*, *l*), one of these (*l*) dying on the same day, two of the others (7, *m*, *n*) dying on the seventeenth day and the fourth (*o*) on the eighteenth day.

This experiment resulted in two races of cells, one of which (from the posterior end) were normal, the other abnormal. The part lost was never regenerated and the abnormality was finally transmitted to the posterior end of the original fragment.

(*d*) *Monster Forms.*—These will be considered together with those of Table III. (see page 53).

(*e*) The three cases of regeneration were nos. 4, 11 and 14. Of these no. 4 was cut during division of a vegetative cell. Within half an hour after the operation the division was completed, one perfect cell and a small imperfect cell resulting. In 24 hours both were living and both were of normal shape but one was much smaller than the other. At the end of 48 hours the small one had grown to a normal cell both in size and shape, but on the third day it was dead.

No. 11 was also a dividing cell when cut through zone 2. On

the following day the larger fragment appeared entirely normal save for an accumulation of crystals at the anterior end (Fig. 10, *a*, *b*); the smaller cell was truncate. On the second day both were normal.

No. 14 was a large vegetative cell which had been treated with neutral red prior to cutting. The smaller fragment, contrary to the usual history, remained alive for six hours but was dead the next day. The larger fragment did not regenerate within 48 hours. The watch glass containing the fragment was not examined again for a period of eleven days when it was found to contain 12 large giant forms and eight small forms normal save for size.

Analysis of Table III.

The cells cut in zone III. gave a higher percentage of living fragments than those cut in zone II. 48.8 per cent. continued to live after 24 hours as against 42.6 per cent.; while 36.6 per cent. continued to live after 48 hours as against 20.6 per cent. The percentage of monsters in both zones was about the same, viz., 12.2 per cent. and 13.2 per cent. but there was not a single case of regeneration. Of those that lived but did not regenerate, some (3) were cells just after conjugation; others (2) were conjugating when cut, while the remainder were large vegetative forms. The reactions for the most part were similar to those of cells cut in zone II., the conjugating forms, or those just out of conjugation, giving the only novel results. These were nos. 1, 2, 3, 4, 8 and 9 of Table III. In nos. 1, 3 and 4 the cells were ex-conjugants when cut, the fragments being killed after 96 hours for the determination of the nuclear condition. Fig. 12 shows the structure of nos. 1, 3 and 4 at this period, the characteristic fragmented nucleus showing a terminal stage of conjugation. It is possible that these cells would have continued to live and to divide had they had a longer time, for they were active and normal in appearance when killed. It was quite otherwise, however, with cells cut during conjugation (nos. 2, 8 and 9). In all cases the fragments died within four days without any attempt at regeneration. In one case (no: 8) the cells were still united but dead after 24 hours; in another (no. 9) both lived for several days without regenerating or dividing; in another case (no. 2) they separated and lived for 48 hours when both died.

Of the vegetative forms the most interesting cases were nos. 7 and 19, the former typical of the majority of cases, the latter unusual. No. 7 was a small form cut just below the mouth. It grew very large but did not regenerate nor divide in the four days of observation, and was finally killed. The nucleus was normal although many fragments of macronuclear material indicated that the original cell was a recent ex-conjugant and was in about the fourth generation when cut.

No. 19 was more interesting. It was cut just below the mouth as in the preceding case. In 24 hours it had grown very large and had divided through the original center of the cell, forming one normal and one truncated cell (Fig. 9, Plate I.). The normal cell divided again before the third day forming two perfectly normal paramecia which were then discarded. The truncated cell grew very large before the third day and divided on the fourth day into one small abnormal cell (Fig. 9, *b*, *c*, *e*) and one larger normal cell (Fig. 9, *d*). On the fifth day the larger, normal form had divided while the small truncated one had developed a sharp posterior end and was almost normal in appearance; finally on the tenth day, all were living and all normal save for a noticeable difference in size (Fig. 9, *g*, *h*, *i*, *j*). Here, therefore, there was a gradual return, through four or more generations to the normal form, although the single cell failed to regenerate.

Analysis of Table IV.

Cells cut in the extreme posterior end were seriously damaged although the abnormalities were not so marked as in the other experiments and 10 per cent. of them regenerated. Only 40 per cent. were alive after 24 hours and only 20 per cent. after 48 hours showing that, although not much mutilated, vitality was nevertheless badly affected by the operation. There were no monsters, although in one case (no. 7) the cell divided 24 hours after cutting but the daughter cells had great difficulty in separating, remaining attached by a narrow isthmus of protoplasm for one full day and finally separating. Within a few hours after separation, however, both cells died. This remarkable effect was produced by cutting off only the most posterior tip of the original cell. It seems hardly credible that so marked an

effect can be produced by such a minor mutilation and it suggests the possibility pointed out by McClendon, of different electrical states at different portions of the cell.

Again, in no. 12 the fragment lived for five days without division or regeneration and finally died. Peristome and mouth were normal but the loss of the posterior end appeared to be fatal.

No. 15 divided 24 hours after cutting forming one truncated posterior cell and a normal anterior cell. The physiological balance, however, was again disturbed for both cells died on the following day.

No. 16 was not so seriously affected by the operation; the cut fragment grew larger each day up to 72 hours when it divided into one truncated and one normal cell, the latter dividing again on the fifth day, the former dividing on the sixth, forming practically normal cells.

Of the forms that regenerated (nos. 1 and 3) both were conjugating when cut. In no. 1 both cells were cut, separation followed normally and each cell had filled out normally by the following day. One, however, grew more and more feeble until it finally died. The other was entirely normal and was used for experiment 21. In no. 3 only one cell of the pair was cut, the other remaining uninjured. After 24 hours both were perfect in form, although one was perceptibly smaller than the other. Both died on the sixth day.

Monster Forms.—Of the 13 monsters only one resulted from a specimen that was cut during division, all of the others came from fragments of vegetative cells. Eight of the thirteen were cells that had been cut in zone II., the other five in zone III. In all cases the power to divide was limited to the nucleus, the cell body apparently being incapable of division.

In no. 6 (Table II.) the cell was dividing when cut through the anterior half of the posterior cell (Plate III., Fig. 18). The fragment lived for three days when it was killed for nuclear structures. Twenty-four hours after cutting the posterior part developed a new peristome and mouth on the cut surface, but the original division was never completed. When killed the cell had one large nucleus which had grown during the three days. The other nucleus had apparently been cut away.

No. 6 (Table III.) was a similar monster, but was derived from a normal vegetative cell cut in zone III. After three days the fragment had attempted to divide; a constriction was present but the posterior cell was only a swelling with a mouth opening and no peristome (Plate III., Fig. 19, *a*, *b*, *c*).

In the remaining eleven cases of monster formation many degrees of malformation resulted from the operation. Of these the least monstrous was no. 23 (Table III.). The original cell was cut as shown in Fig. 13, Plate II. There was no sign of regeneration during the next three days but the cell grew larger and finally attempted to divide (Fig. 13, *b*, *c*). One of the cells was normal in shape and size; the other, posterior, was smaller and truncated. The two remained attached for a period of three days swimming about actively, bending and twisting with the various cilia in action, until they finally died, undivided, nine days after the operation. The nuclear apparatus was not determined. In this case two complete peristomes and mouths were developed and the cells were attached by only a delicate strand of protoplasm. The general result was similar to that of experiment no. 7 (Table IV.) in which the daughter cells remained attached for 24 hours and finally separated, dying shortly after the separation (see page 52).

Other monsters formed without division of the cut cell were nos. 35, 39, 40, 51 and 68 of Table II., and nos. 11 and 41 of Table III. Nos. 35 and 39 were killed after 4 and 5 days respectively, while nos. 68, 11 and 41 died in from 4 to 8 days. The other two, nos. 40 and 51, lived for 20 and 17 days respectively, and developed into relatively huge protoplasmic masses.

The remaining three monsters, nos. 22 and 25 of Table II., and no. 17 of Table III., all came from an original form that divided after the operation into one normal and one abnormal cell, the monster in all cases coming from the abnormal cell.

The history of only the most interesting of these monsters need be given, all matters of importance in the other cases being included in the history of these.

The simplest case is no. 51 which lived, after cutting, for 17 days undergoing two attempts to divide in that time. The cell was cut as shown in Fig. 14, Plate II., in zone II., and the frag-

ment was very lively for 72 hours growing enormously during that period (Fig. 14, *b*). Between the third and the fifth day it divided giving rise to a monster as shown in Fig. 14, *c*. The mass had two peristomes and two mouths placed as shown in the figure, and continued to grow in size changing form from day to day and adding a new mouth on the sixteenth day. It died on the seventeenth day. The nuclear apparatus had disintegrated with death of the mass and could not be determined. This case gave the largest mass of protoplasm obtained, with the fewest cell organs, only one contractile vacuole being observed and with only two mouths for most of the time. The power to grow was intact and the mass great enough for six cells.

No. 17 (Table III.) is another simple case of monster formation which differed from the foregoing in appearing after the first division subsequent to the operation. The cell was cut in zone III. as shown in Fig. 15, *a*. On the following day the fragment had divided into an abnormal and a normal cell (*b*, *c*, *d*). On the second day the normal cell divided while the abnormal one remained as before save for growth. On the third day the normal cell divided once again into two normal cells, and the abnormal one attempted to divide but formed a monster with a small anterior and a larger posterior portion. There were two mouths, two peristomes and two contractile vacuoles. On the fourth day the monster had changed somewhat in axial relations so that the forms of two paramecia could be made out. The mass however was weaker than on the preceding day and died on the following (fifth) day. A preparation showed the presence of two macronuclei and two micronuclei in their appropriate positions (Fig. 15, *e*).

Experiment 40 (Table II.) gave one of the most interesting of the monsters. The cell was cut in zone II. on the fourth of February. Forty-eight hours later it had attempted to divide in the original center of the cell but formed a monster with a small anterior cell and a full size posterior cell (Fig. 17, *a* and *b*). Two complete peristomes with mouths were present and two contractile vacuoles. These conditions were retained without further morphological change save growth in size until February 10, or the sixth day, when a drop of nuclein was added to

the ten drops of hay infusion medium. On the seventh day the monster had grown enormously (Fig. 17, *c*); on the ninth day it gave rise to a small free cell which was an exact miniature copy of the original cut fragment (17, *e*). This cell arose from the upper end of the monster as shown in 17, *d*. On the tenth day the isolated fragment was killed for the determination of the nuclear apparatus and was found to have a normal full size macronucleus. On this day also, the monster divided at the point *x* forming two monsters, one having three mouths (17, *g*) the other with only two (17, *f*), but the latter at the time of division, was also dividing to form a small terminal deformed cell. Thus two divisions were going on at the same time in the protoplasmic mass. The small deformed cell was detached from the parent monster on the same day and appeared as a minute reversed duplicate of the original fragment (Fig. 17, *h*). This, like the first detached fragment, had a single contractile vacuole. On the eleventh day the small fragment was still alive and active but had not regenerated nor grown in size while the two monsters had grown to look more like paramecia fused (17, *i* and *j*). On the twelfth day the small fragment was dead while the others remained as before. On the thirteenth day the smaller monster had grown much weaker and was killed on the eighteenth of February or the fourteenth day (17, *k*). The remaining monster was very plastic changing shape from day to day until on the twenty-fourth of February appearing very weak, it was killed, twenty days after the original operation during which time no more than three mouths were formed in this part of the divided monster. The original fragment thus developed seven mouths indicating at least seven attempts to divide while the monster divided once to form two monsters. Preparations (17, *k* and *l*) show relatively enormous nuclear masses.

One interesting feature of this experiment was the formation of free-living but abnormal fragments separated off from the protoplasmic mass. In experiments nos. *22* and *25* (Table II.) the monsters gave rise to similar cells either attached (*22*) or free (*25*), but in every case these offshoots were normal in structure although abnormal and weak in function.

In No. *22* the cell was cut in zone II. as shown in Fig. 16, *a*,

leaving the lower part of the peristome, the nucleus and the mouth intact. Twenty-four hours later the truncate cell divided in the plane of the original center into a small truncated anterior fragment (*b*, *c*, Fig. 16), and a normal cell (16, *d*). The abnormal fragment had one contractile vacuole, the normal cell two. Seventy-two hours after the original cut the normal cell (*d*) divided into two perfectly normal paramecia, which divided again on the following day into normal forms, thus establishing the entirely normal condition of this product of the original truncate fragment. The abnormal fragment (*c*) neither regenerated nor divided for the first five days after the operation, although it grew considerably in size (16, *e*). On this fifth day, however, it attempted to divide, but cytoplasmic division failed and a monster with two mouths, two contractile vacuoles and two blunt ends was formed (16, *h*). The power of division seemed to be again restored for on the sixth day the mass attempted a second division, and this time a perfect *Paramecium* was formed, but, as in experiment no. 7, Table IV., it could not separate from the parent mass and remained attached by a delicate connection throughout life of the monster (Fig. 16, *i*, *j*). On the seventh, eighth, and ninth days the monster gradually assumed the indefinite outlines of three paramecia (Fig. 16, *i*) but when finally killed on the ninth day these outlines were again lost (*j*). The macronuclei were properly distributed for three individuals but the micronuclei were increased in number, six perfect ones being found, separated by considerable distance from the macronuclei.

In experiment no. 25 not only were normal individuals formed but they were detached and lived for some days as ordinary paramecia. The original vegetative cell was cut in zone II. on January 28, leaving the nucleus, the lower part of the peristome and the mouth intact (Fig. 20, *a*, *b*). On the twenty-ninth the fragment divided in the original center of the cell forming a small truncated anterior fragment and a normal posterior cell (Fig. 20, *c*, *d* and *e*). The normal cell, as in experiment 22 above, divided on the following day forming normal cells, and these continued to divide normally on the average of once a day throughout the life of the other fragment and were discarded on death of the latter. The anterior truncate cell (*e*) grew

much larger during the 48 hours after the operation and the 24 hours after division, and attempted to divide on the thirtieth, the same day that its normal sister cell divided. The result of this effort was a monster with two mouths and two vacuoles (Fig. 20, *f*). On the first of February, or the fourth day after the operation, the monster attempted a second division, this time a double division, the result being one free individual and a monster with three mouths (Fig. 20, *h*, *g*). This free individual was smaller than the normal but it lived for 48 hours and died during the process of division. It was detached from the parent mass at the point corresponding to the upper end of Fig. 20, *g*. The monster, meanwhile, continued to grow in size and on the sixth day had two perfect *Paramecium* buds attached by their posterior ends, and several papilla-like outgrowths which continued to grow like buds until on the ninth day well-defined *Paramecium* cells were formed but remained attached to the mass of protoplasm (Fig. 20, *i*, *j*). On the ninth day a second normally formed cell (*k*) was detached, this being the upper one figured in 20, *i*. This free form did not attempt to divide but died before the end of 24 hours. On the twelfth day, still another free form was given off (Fig. 20, *n*), this being the individual on the left side of Fig. 20, *l*. This one was small like all of the detached forms, but appeared to be normal, living for 36 hours. On the tenth and eleventh days the outlines of the previously distinct paramecia buds on the parent mass, became obscure and the individuals seemed to fuse with the protoplasmic mass while the latter twisted and turned with the various ciliary movements. At one period on the tenth day the mass became drawn out into two main lobes (*l*) with a connecting strand between them and appeared to be dividing, but the figure was due to the mechanical pressure caused by the presence of a girdle of zoöglœa about the middle. When this girdle was removed with needles, the protoplasm returned to the more solidly massed state (*m*). A similar constriction appeared on the thirteenth and fourteenth days and zoöglœa was once more removed and the obscure outlines of ghostly paramecia again appeared, no less than eight bizarre forms stretching out from the parent mass at one time while six more mouths could be counted on the general surface (Fig. 20, *o*).

On the following day or fifteenth day after the operation, two dead masses of protoplasm were found; the monster (Fig. 20, *o*) had divided in the middle and the two separated masses evidently had not been physiologically balanced, and had died.

In preparations made with the dead masses no definite nuclei could be distinguished, the material had evidently died during the night and had been dead too many hours for the preservation of the nuclei. A large granular mass in one of the fragments may have been the remains of nuclei perhaps aggregated into a mulberry mass as described by Balbiani.

General.

Balbiani's conclusion that *Paramecium* does not regenerate as do other protozoa is too sweeping a generalization. A small percentage of cases do regenerate, but the percentage varies with different races. In the experiments here outlined, three different giant races of *Paramecium caudatum* were used. One race, from brackish water in Roscoff, gave approximately 10 per cent. of regenerations; another race, from New York, gave approximately 1 per cent.; another race, also from New York, gave a relatively high percentage of regenerations, approximately 30 per cent., and in a fourth race now under examination every one—or 100 per cent.—regenerates. The method employed by Balbiani was to cut *en masse* instead of individuals one by one. This method made it impossible to determine twenty-four hours afterwards, whether the perfect cells had not been cut at all, or had been cut and had regenerated.

From my results it appears, therefore, that different races have different powers of regeneration, or, stated in another way, have varying degrees of vitality. This power of regeneration is connected in some way, apparently, with the physical make-up of the protoplasm. In other forms of protozoa, notably in *Stentor* (many observers, including Balbiani, Gruber, Nussbaum, Verworn, Prowazek, Lillie, Popoff and others), in *Loxophyllum* (Holmes), in *Stylonychia*, *Oxytricha*, *Spirostomum*, *Frontonia*, *Trachelocerca*, *Dileptus* and *Spathidium*, all of which, save *Loxophyllum*, I have experimented with in this connection, the protoplasmic walls come together more or less quickly after the operation of

cutting, thus leaving no endoplasm exposed. In *Stentor*, *Spirostomum*, *Trachelocerca*, *Spathidium*, *Dileptus* and *Frontonia*, the walls come together in a relatively short time, but in *Stylonychia* and *Oxytricha*, the union is delayed and sometimes never occurs. In *Paramecium*, on the other hand, with the exception of the fourth race mentioned above, the cut fragments form a new membrane upon the cut surface and the normal form is not regained, the cell dividing asymmetrically. Where the vitality is sufficiently strong for regeneration the normal form is occasionally gained before division of the fragment, but more often after a gradual change requiring several generations. In the fourth race, on the other hand, the walls of the fragments behave exactly as in *Stentor* or *Loxophyllum* and regeneration is perfect.

The difference in regenerative power may be due to the differences in potential at different periods of the life cycle, and correlated with differences in the physical make-up of the protoplasm at such periods. This certainly appears to be the case with the power to divide, abnormal divisions or monster formation taking place at the end of a long series of generations (see Calkins, 1904), and in those races of *Paramecium* where regeneration occurs in a small percentage of cases.

Child and others have concluded that movements have much to do with the restoration of form in regenerating animals. Holmes shows that this is of secondary importance in the regeneration of cut fragments of *Loxophyllum*. So too in *Paramecium*, movement apparently plays no part whatsoever in the restoration of form. The old peristome is always the site of the new mouth formation, but in the majority of cases, if the mouth is cut away, the anterior fragment with the bulk of the peristome fails entirely to form a new mouth. The general shape of the cell is usually retained; there is no rounding out of the cut fragment any more than there would be were the *Paramecium* made of cheese. Form, therefore, is a highly stable characteristic of *Paramecium*. This is demonstrated with remarkable clearness in the case of monsters. A two mouth monster is, at first, little more than an elongate amorphous mass of protoplasm (Figs. 14, 15, 20). As it grows older, however, the typical shape of the organism begins to appear. Still more remarkable is the budding

out of individual forms in monsters of older growth. Here, as the mass is watched from day to day, fairly complete cells make their appearance only to be absorbed again in the general mass. This absorption is analogous to that resulting from a wounded *Paramecium* in which the cut failed to extend entirely through the diameter of the cell. In such cases the wound is closed and the cut surfaces fuse within a short time. Experiment no. 25 (Fig. 20) is a good illustration of the appearance and disappearance of individuals from the common protoplasmic mass of such a monster, the buds always growing into a semblance at least of the typical *Paramecium*.

Form in *Paramecium*, therefore, is a persistent characteristic, apparently independent of movement, and connected in some way with the physical make-up of the protoplasm.

Regeneration of the organism which occurs in a small percentage of cases varying in different races, is probably due to a perfectly balanced physiological condition of the cell. This conclusion is deduced from the fact obtained in many experiments, that a comparatively insignificant cut whereby only an extremity of a *Paramecium* is removed, has a profound effect upon the further activities of the cell. Examples are given in experiments of Table I. and of Table IV., where one or the other end of the cell was removed. In the majority of cases the residual fragment died within a short period; in a few cases the residual fragment divided asymmetrically, giving rise to one normal *Paramecium* which continued to live and to multiply normally, and an abnormal cell which soon died; in a few cases again, the fragment regenerated either before or soon after division.

If this phenomenon could be explained we should be well on the way to an explanation of that ghost-train of processes which we designate vitality. To speak of a balancing of processes, or of a stable and unstable equilibrium, however, is no explanation of what takes place. Some recent observers have undertaken the task of explaining cell physiology, including division, through the varying ratio of nuclear mass to cytoplasmic mass. Balbiani believed that monsters are due to some slight injury to the macronucleus in cutting, and made the generalization that they are "always individuals mutilated in the anterior part whose de-

scendants give rise to abnormalities. . . . Those which lose the posterior part only, always multiply normally by fission. . . . A sort of paralysis attacks cells thus injured in their nuclei" ('92, p. 78).

The number of monsters (four) obtained by Balbiani is too small to support such a generalization. My experiments, in which five of the thirteen monsters were derived from cells cut in the posterior half throw this suggestion out of further consideration.

A more modern view of the nature of physiological processes of the cell was suggested by R. Hertwig ('03) and has been elaborated by himself and his school in numerous publications. This theory according to Popoff's ('08) presentation, involves the view that the quotient obtained by dividing the cytoplasmic mass by the nuclear mass is fairly constant under "normal" conditions, and just as long as this relation varies only within narrow limits, the cell functions are normal. But if by disproportionate growth of either cytoplasm or nucleus, the nucleus-protoplasm relation is changed to favor either one, then the cell gets into an "abnormal" condition. In order to become "normal" again, the "normal" nucleus-protoplasm relation must be reëstablished. Cell division, Hertwig believes, is the means whereby normal relations are reëstablished. He postulates, furthermore, two periods in the growth of the nucleus; one "functional," the other "divisional," the former beginning shortly after division of the cell and lasting until shortly before the following division, the nucleus growing less rapidly than the cytoplasm and disturbing the nucleus-protoplasm relation in favor of the cytoplasm. This disturbance of "normal" relations persists up to a certain point which Hertwig calls the nucleus-protoplasm-tension-moment (Kernplasmaspannungsmoment), which he regards as the immediate inciting cause of cell division, the first effect being the rapid "division growth" of the nucleus. The result of the division thus started is a return to the "normal" nucleus-protoplasm relation.

The use of terms normal and abnormal in this connection is hardly appropriate, for cell division is certainly a normal process and all stages leading to it must likewise be normal, hence an

abnormal nucleus-protoplasm relation in a normal vegetative cell, cannot exist. Waiving this verbal matter, however, and examining the principle involved, we admit the change in the relative masses of nucleus and cytoplasm in periods of growth. As to the growth of the nucleus prior to cell division the evidence is not so clear. In *Uronychia transfuga* at division, for example, not only is the exposed surface of the nuclear elements lessened but the mass as well is decreased, and the two small cells resulting from the division contain small nuclei which subsequently break up into fragments before growth commences and with growth there is a constantly increasing surface of nuclear matter (Calkins, '11). On the Hertwig theory we should expect a restitution by division, of the "normal" mass relations in fragments of cells containing either a preponderance of nuclear matter or of cytoplasmic. Fragments of cells, however, show no such regulatory process, for cutting the cells invariably postpones division unless the division is already under way. Under abnormal conditions of temperature Popoff finds that the volume of the nucleus increases more rapidly than that of the cytoplasm. Various observers have noted that under abnormal conditions, generally, including that of weakened vitality from any cause, the nucleus is relatively larger than under normal conditions and the enlargement is better interpreted as an effect of lessened vitality than its cause. Popoff likewise performed cutting experiments on *Frontonia leucas* and found, as Balbiani and others have found, that the cell, if cut late in an inter-divisional period, divides in the original plane of division. But if the cell is cut before this period the cell first regenerates before dividing. He concludes: "Diese Versuch zeigen, dass der Anstossgebende Moment der Theilung in dem Augenblick der Kernplasmaspannung zu suchen ist. So wie die Zelle diesen Moment uberschritten hat, ist der Teilung derselben schon eingeleitet und kein ausserer Eingriff kann sie mehr verhindern" ('08, p. 337). It is difficult to see this conclusion from the facts presented and still more difficult to interpret such cases as my experiment 19, Table III., where two successive divisions of a fragment were entirely asymmetrical (see p. 52). In this case furthermore there was a long period between divisions when

"normal" conditions of the division energy might have been reëstablished.

In reference to my *Paramecium* work of 1904 Popoff states that at the final period of depression the nucleus-protoplasm relation was changed to the advantage of the nucleus and he argues that this condition may have been the cause of the depression (*loc. cit.*, p. 339). As my paper of 1904 makes no reference to the nucleus-protoplasmic relation he drew his conclusion from the photographs of the cells at the end period of depression, but naturally, he did not realize that when the cells thus photographed were killed, they had lived for days without dividing. Under such conditions, like any other *Paramecium* cell under similar conditions, they were "abnormal" so far as the nucleus-protoplasm relation is concerned.

In the latter part of the first section of Popoff's paper of 1908 he gives an inkling of a possible interpretation of the nucleus-protoplasm relation in the statement: "Die Kernplasmarelation wird ein morphologischer fassbarer Ausbruck der jeweiligen Chemismus der Zelle bleiben." With this interpretation we are inclined to agree, and we look upon the changeable nucleus-protoplasm relation as an unstable effect produced by varying conditions of nutrition, temperature, or vitality and not at all as a cause of division or depression or of vitality. In a mutilated *Paramecium* the nucleus divides equally, the cell unequally; the smaller fragment has a full size nucleus and a much deranged "nucleus-protoplasm" relation; yet, in some cases at least, it behaves like a normal cell, dividing at the proper time and again forming dissimilar products, one of which is perfectly normal, the other again abnormal (expt. 25, p. 57).

By removing a portion of a cell there can be little doubt that the ordinary chemical interchange, or perhaps the physical or electrical potential, of cell and nucleus is violently disturbed. In forms with a labile protoplasm, as manifested in some forms of *Paramecium* and as in *Stentor*, *Loxophyllum Spathidium*, etc., the wounded cell regenerates quickly, but in other races of *Paramecium* and in non-nucleated fragments of other protozoa, the more stable protoplasm does not respond and regeneration fails. Division of the fragment indicates that the power of

division and the power of regeneration are entirely independent phenomena and are alike independent of the nucleus-protoplasm relation so far as mass is concerned. The failure of a small fragment to divide completely might be due to the fact that the division energy is not great enough to overcome the surface tension of the body walls. But this interpretation would hardly account for the incomplete division of the cell in which only a small portion of the terminal protoplasm is removed (experiment 7, Table IV., p. 52).

SUMMARY.

1. *Paramecium caudatum* may be easily cut with a scalpel, one, the larger, part, continues to live in about 30 per cent. of cases, and both parts never for more than 24 hours. If the nucleus is injured by the knife neither part lives for more than a few hours. The present paper is a summary of about 150 recorded experiments.

2. Cells cut at either extremity are just as much demoralized as those cut in apparently more vital parts.

3. The power of regeneration varies in different races of "giant" *Paramecium*. In one race only about 1 per cent. regenerated; in another race about 10 per cent., in a third race about 30 per cent. regenerated, and in a fourth race all, or 100 per cent. regenerated. This last race is not included in the present paper.

4. There is strong evidence of a division zone in *Paramecium* which lies in the center of the cell. If the cell is cut anterior or posterior to this zone the fragment divides in the original plane into a truncated abnormal form and a normal form. The truncated form may divide again not through its center, but through the center of the cell were it perfect.

5. A fragment, whether anterior or posterior, dies without division in the majority of cases; in cases of fission, it divides asymmetrically as stated above. The division, however, is retarded. In a few cases the division is abortive and a monster results.

6. After such a division into an abnormal and a normal cell, the normal cell continues to divide normally and forms a race of

individuals entirely unaffected by the operation. The abnormal cell in some cases, divides again asymmetrically and forms another normal cell and an abnormal cell; in other cases the second division is abortive and monsters are formed; in a few cases it continues to divide with a gradually decreasing abnormality until normal forms are regained; in many cases, finally, it dies without further division.

7. The monsters represent as many individuals as there are mouths. In one monster as many as fourteen mouths were present at one time. There is a well-marked tendency of the protoplasm to assume the form of a normal *Paramecium* about each of the mouths, and such individuals bud out of the protoplasmic mass, remain for several hours, and may be absorbed again into it.

8. Free cells, complete in all respects, may be given off from the protoplasm of a monster. These may live for days and may even divide, but vitality is weak and they invariably die.

9. Cell division and cell regeneration are entirely independent phenomena. A fragment divides without regenerating and the abnormal product of this division may divide again without regenerating. In other cases the fragment divides asymmetrically and the resulting abnormal cell regenerates before the following division. The cell need not regenerate to divide, and may or may not regenerate before dividing.

10. The *Paramecium* cell acts as a unit; cytoplasm and nucleus are equally important, a small loss at either extremity is usually enough to throw the physiological mechanism out of gear and lead to death, to asymmetrical division, or to monster formation.

11. The phenomena resulting from cutting *Paramecium* cannot be explained on the Kernplasmarelation theory of the Munich school. Mass of nucleus in relation to mass of protoplasm is only a morphological index of the physiological (chemical, electrical) activity of the cell—an effect and not a cause of the various vital reactions.

12. Regeneration of a cut cell in races with a limited power of regeneration, is more often observed in cells that have recently conjugated, or in cells that were cut while in conjugation, than in ordinary vegetative cells.

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Description of Plates.

Unless otherwise stated, all figures are made from camera drawings or sketches from the living cells. The black lines indicate the planes of cutting. All figures are of *Paramecium caudatum*.

Plate I.

Fig. 1. *a*, vegetative cell cut in zone 1; *b*, the fragment at the end of four hours. It died within 24 hours. Experiment 11, Table I.

Fig. 2. *a*, a similar cell cut as above; *b*, fragment 72 hours afterwards showing neither regeneration nor growth. Experiment 4, Table I.

Fig. 3. *a*, conjugating *Paramecium* cut in zone 2; *b*, fragment 24 hours after the operation; *c*, the other, normal, exconjugant; *d*, the fragment fixed and stained after 96 hours, showing normal vegetative nuclei indicating that conjugation had just begun when cut. Experiment 2, Table II.

Fig. 4. *a*, vegetative cell cut in zone 2; *b*, the fragment 6 hours after cutting; *c*, the fragment dividing 24 hours after cutting and killed during division to show the nuclear apparatus. Experiment 7, Table II.

Fig. 5. *a*, vegetative cell cut in zone 2; *b*, fragment dividing, 16 hours later; *c*, anterior, and *d*, posterior, cells killed 24 hours after division for the nuclear structures. The disintegrated fragments of macronuclei indicate recent conjugation. Experiment 9, Table II.

Fig. 6. *a*, vegetative cell cut in zone 2; *b*, fragment 24 hours after cutting, with tentacle-like process; *c*, fragment dividing in the original center of the cell 48 hours after cutting; *d*, *e*, resulting daughter cells 6 hours after division. Both of these cells died on the following day. Experiment 21, Table II.

Fig. 7. *a*, vegetative cell cut in zone 2; *b*, fragment 72 hours after cutting; *c*, fragment in division on the fourth day; *d*, *e*, daughter cells resulting from this division; *f*, cell *d* on the sixth day, much grown; *g*, *h*, daughter cells of *f* on the ninth day; *i*, *j*, daughter-cells of *h* on the twelfth day; *k*, *l*, daughter cells of *g* on the twelfth day; *m*, *n*, figures of *i* and *k* on the seventeeth day when they died; *o*, figure of *j* on the eighteenth day after the operation when it, too, died. Experiment 54, Table II.

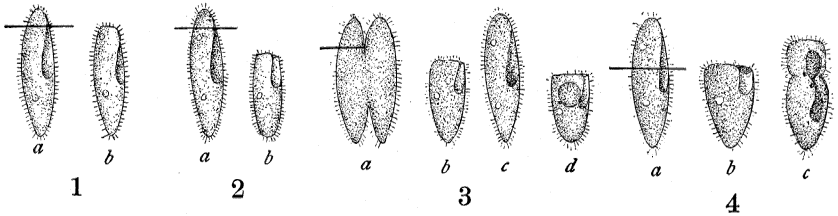
Fig. 8. *a*, vegetative cell cut in zone 2 near the plane of division; *b*, division of the fragment 24 hours after cutting; *c*, *d*, resulting cells, the former minute, the latter normal. Both died on the second day. Experiment 29, Table II.

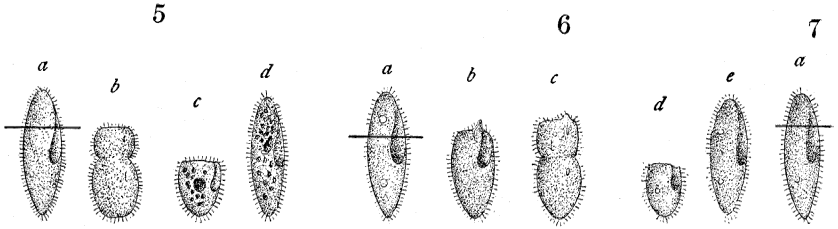
Fig. 9. *a*, vegetative cell cut in zone 3; *b*, fragment 12 hours after cutting; *c*, same dividing 24 hours after cutting; *d*, *e*, daughter cells resulting from division of *b*; *f*, division of truncated cell *e* on the fourth day; *g*, figure of truncated posterior half of *f* on the eighth day; *h*, *i*, daughter cells of the normal half of *f*, on the tenth day, *j*, figure of *g* on the tenth day. Experiment 19, Table III.

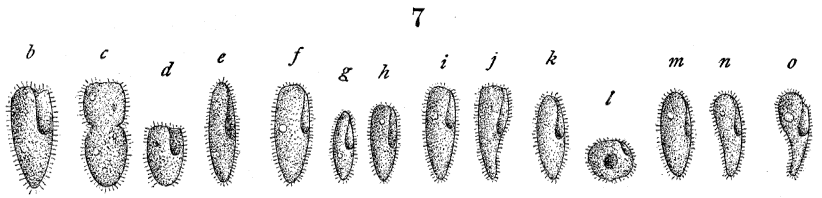
Fig. 10. *a*, dividing cell cut in zone 3; *b*, division figure after removal of the anterior end; *c*, small anterior cell after division; *d*, normal posterior cell after division of *b*; *e*, figure of *c* 24 hours later; *f*, figure of *d*, 24 hours after division of *b*. Experiment 11, Table II.

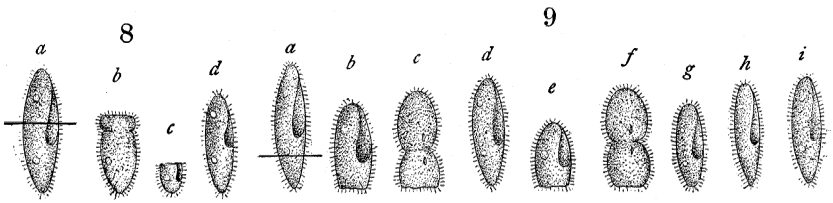
Fig. 11. *a*, dividing cell cut in zone 4; *b*, posterior fragment with mass of crystals in the anterior end. This died on the following day. Experiment 5, Table IV.

Fig. 12. *a*, ex-conjugant cut in zone 3; *b* and *c*, the anterior fragment 48 and 96 hours after cutting.









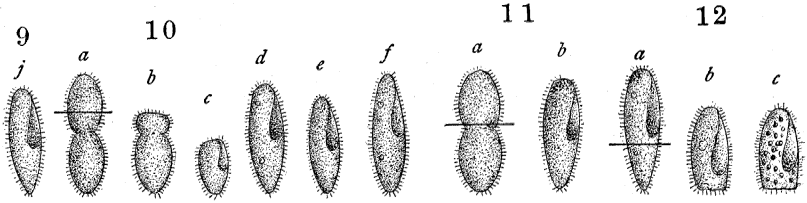


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PLATE II.

FIG. 13. *a*, vegetative cell cut in zone 3; *b*, fragment three days after the operation; *c*, asymmetrical division on the fourth day; *d*, same on the seventh day; *e*, same on the eighth day. Dead on the ninth day. Experiment 23, Table III.

FIG. 14. *a*, vegetative cell cut in zone 2; *b*, fragment 3 days after the operation; *c*, monster from asymmetrical division on the fourth day; *d*, same on the fourteenth day. Dead on the seventeenth day. Experiment 5, Table II.

FIG. 15. *a*, vegetative cell cut in zone 3; *b*, asymmetrical division of the fragment on the following day; *c*, *d*, normal and abnormal cells resulting from the division; *e*, monster formed by the cell *d* on the third day after the operation. Dead on the fifth day with nuclear apparatus normal. Experiment 17, Table II.

FIG. 16. *a*, vegetative cell cut obliquely in zone 2; *b*, asymmetrical division of the fragment 24 hours later; *c*, *d*, abnormal and normal cells resulting from this division; *e*, abnormal cell *c* on the fourth day; *f*, *g*, normal cells resulting from normal fission of *d* on the third day; *h*, monster formed from *e* on fifth day; *i*, second attempted division of the monster on the the sixth day; *j*, same monster after fixation and staining on the ninth day. Experiment 22, Table II.

FIG. 17. *a*, vegetative cell cut in zone 2; *b*, attempted division of the fragment 48 hours after the operation; *c*, same monster on the seventh day, 24 hours after addition of nuclein; *d*, same monster on the ninth day after a second (double) attempted division; *e*, free, truncate fragment derived from the upper extremity of the monster figured in *d*; *f*, *g*, two monsters derived from the division of monster *d* at the middle point (*x*) on the tenth day; in *f* the lower branch is in the process of division; *h*, small, reversed, truncate fragment 24 hours after being formed from division of the lower branch of *f*; *i*, *j*, the two monsters on the eleventh day; *k*, the smaller monster after fixation and staining on the fourteenth day; *l*, the larger sister monster after fixation and staining on the twentieth day after the original operation. Experiment 40, Table II.

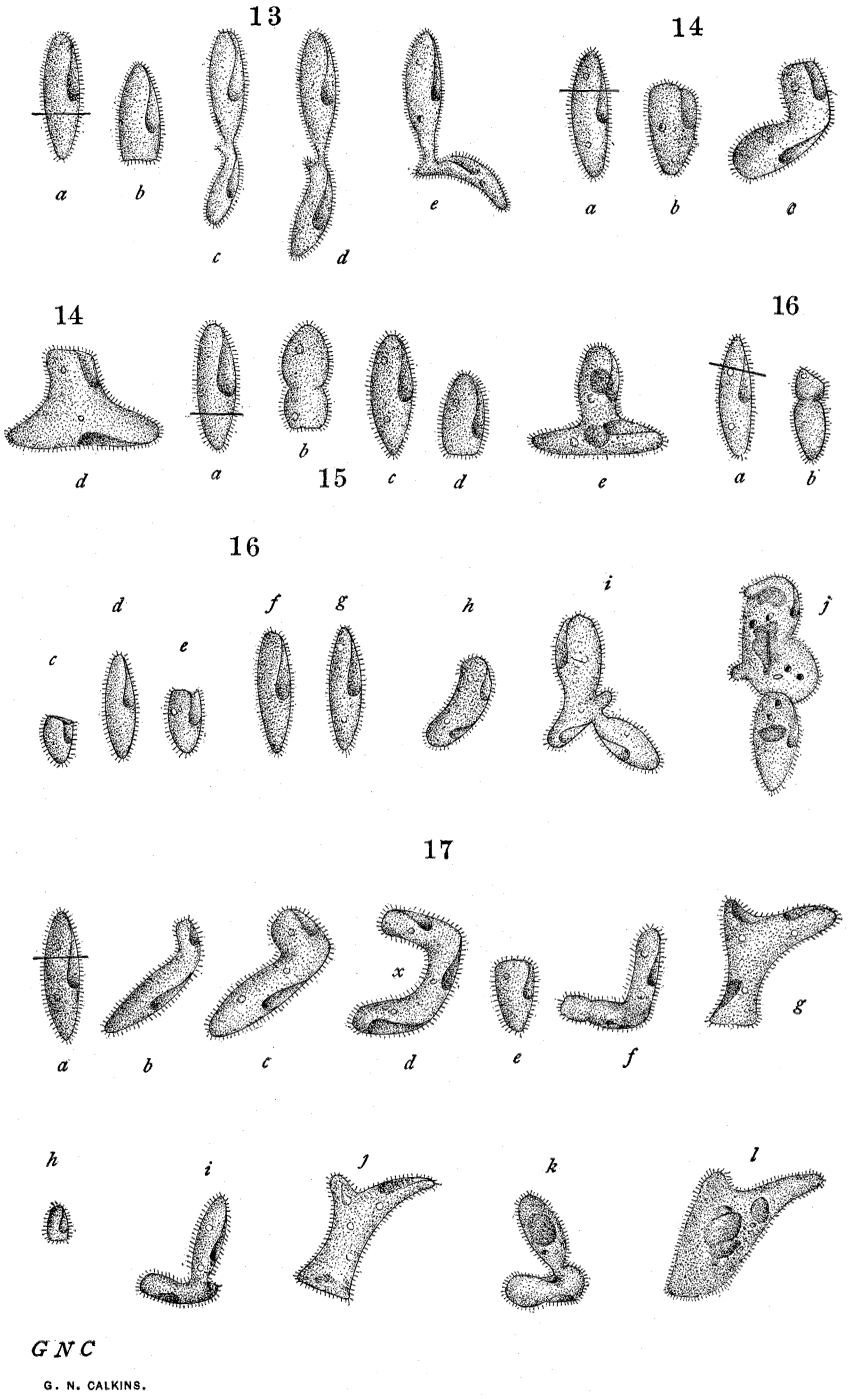

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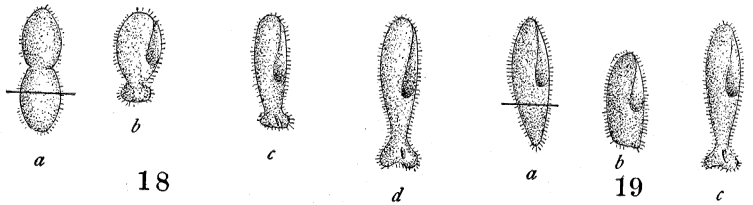
PLATE III.

FIG. 18. *a*, dividing cell cut in zone 2 of the posterior cell; *b*, fragment 24 hours after the operation; *c*, fragment 3 days after the operation. Experiment 6, Table II.

FIG. 19. *a*, vegetative cell cut in zone 3; *b*, anterior fragment 48 hours after the operation; *c*, attempted division resulting in monster formation on the third day after cutting. Experiment 6, Table III.

FIG. 20. *a*, vegetative cell cut in zone 2; *b*, posterior fragment 12 hours after the operation; *c*, asymmetrical division of *b*, 24 hours after the operation; *d*, *e*, normal and abnormal cells resulting from the division of *c*; *f*, attempted division of *e* 48 hours after the operation; *g*, *h*, results of a second (double) division of *f* on the fourth day; *h*, a normal *Paramecium* derived from the upper branch of *g*; this lived for 48 hours and attempted to divide but died in the process; *i*, monster derived from *g* on the sixth day, with two practically complete paramecia and several papilliform buds; *j*, same monster on the ninth day; *k*, a detached free cell on the ninth day derived from the upper cell shown in figure *i*; *l*, same monster on the twelfth day with constriction due to a girdle of zoöglœa; *m*, same monster on the thirteenth day with individuals partially withdrawn; *n*, a third, free individual given off on the twelfth day, this being the attached individual on the left side of Fig. *l*; *o*, same monster on the fourteenth day with eight *Paramecium* buds, six mouths additional (not shown) and ten vacuoles. As the figure shows the mass was again constricted and this time the constriction resulted in division but the daughter monsters were dead on the next day. Experiment 25, Table II.



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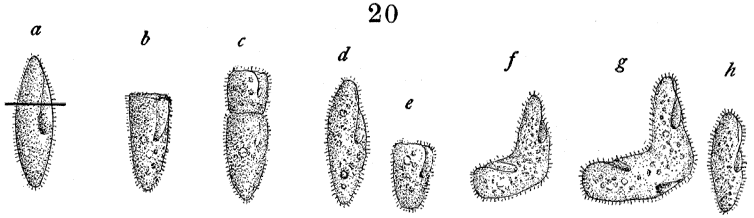

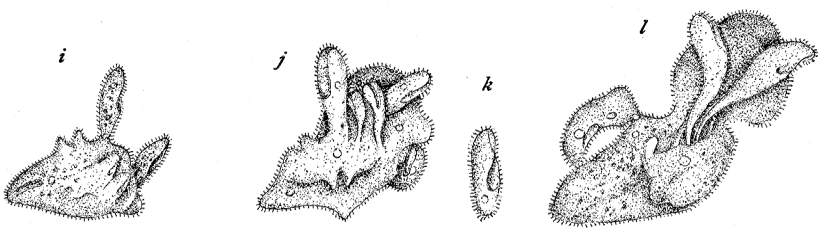

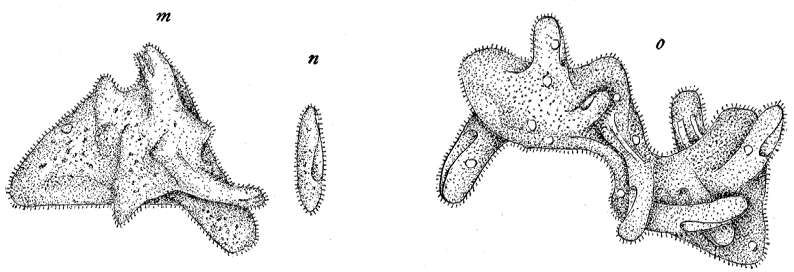

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